

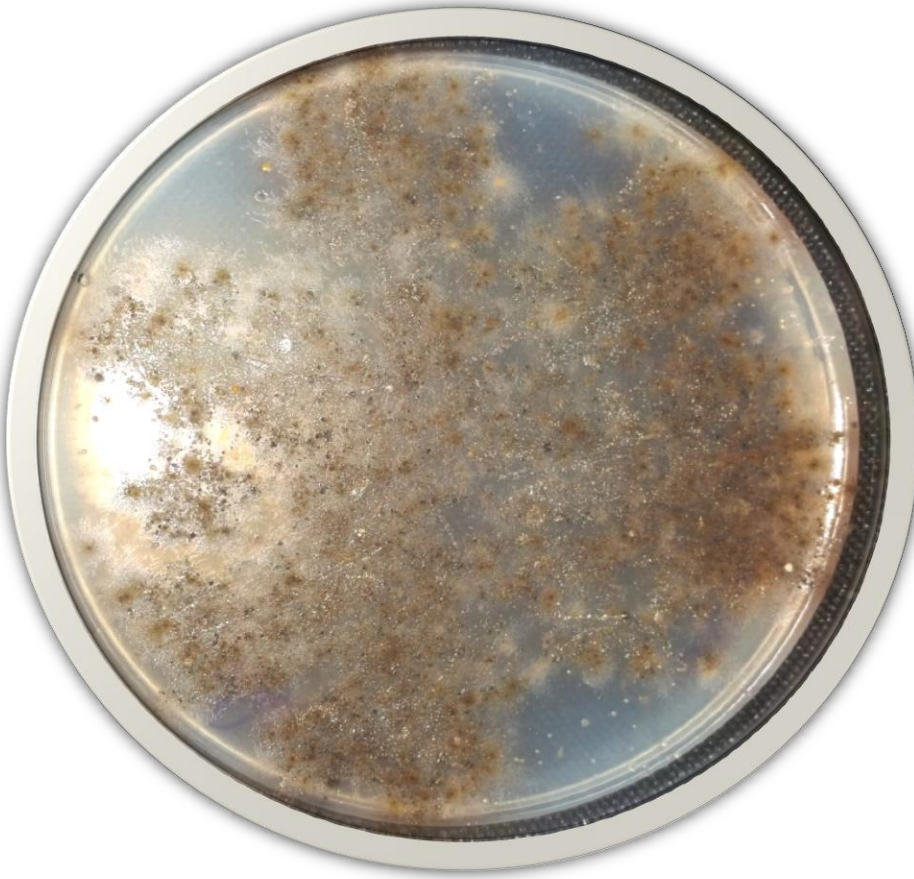
Investigations on ecology and transmission of *Rhodococcus* species in California nut crops

Elizabeth J. Fichtner, PhD
UCCE Tulare and Kings Counties

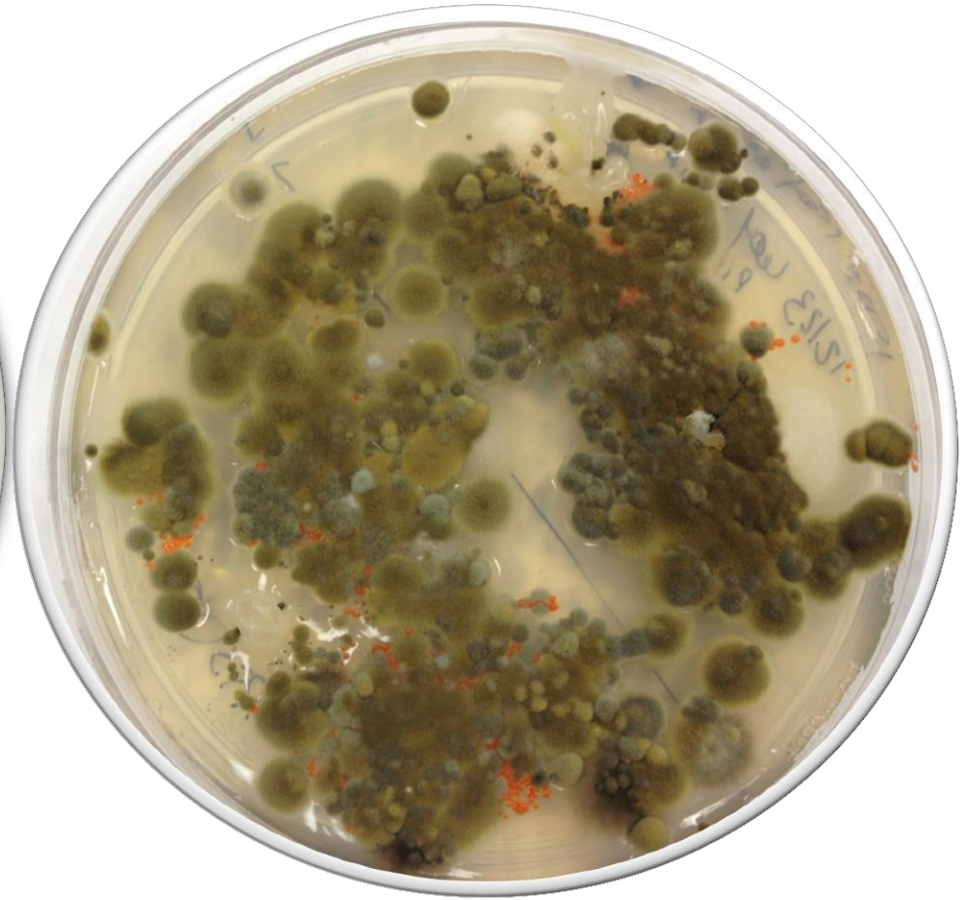
1. Association of *Rhodococcus* with PBTS trees
 - improved detection for diagnosis
2. Replant Disease Risk?
 - field survey of replant roots
 - pistachio root susceptibility
 - infectivity of replant soils
 - almond rootstock susceptibility
3. Pathogen transmission in field
 - transmission on pruning tools
 - other possible modes of transmission
4. Prevalence on other hosts?
 - survey of CA walnuts.



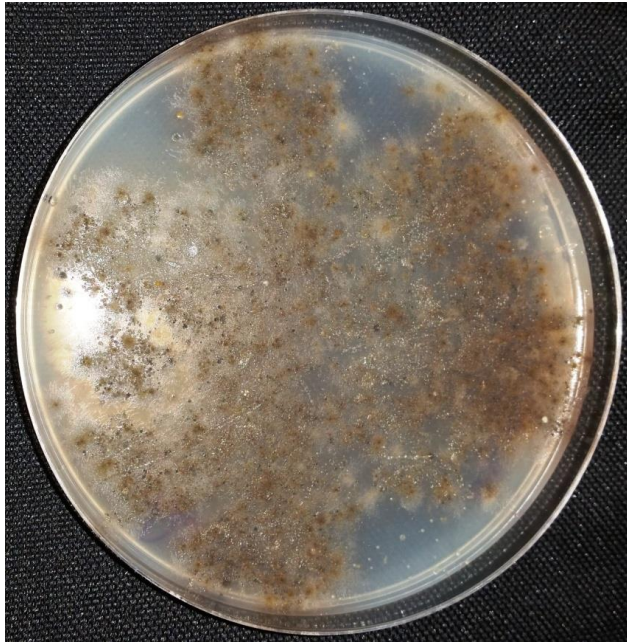
How prevalent are *Rhodococcus* spp. on symptomatic PBTS trees in the orchard?



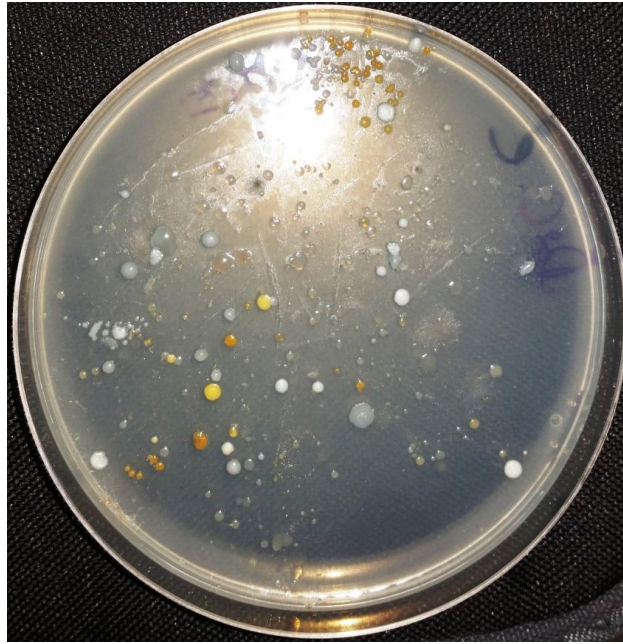
Leaf print from symptomatic PBTS tree on D2 medium.



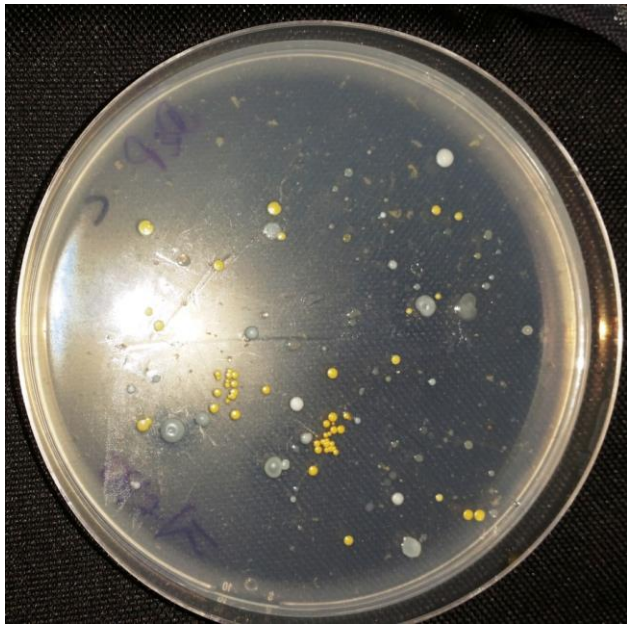
Leaf print from inoculated GH tree on D2 medium.



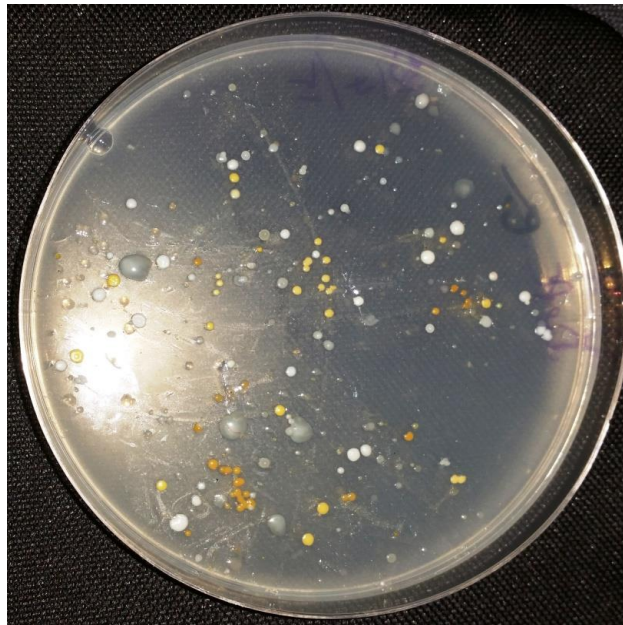
D2



D2+Cycloheximide



D2+Pimaricin

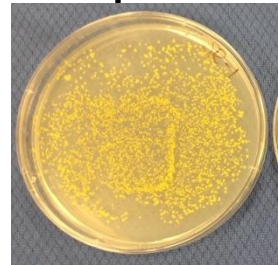
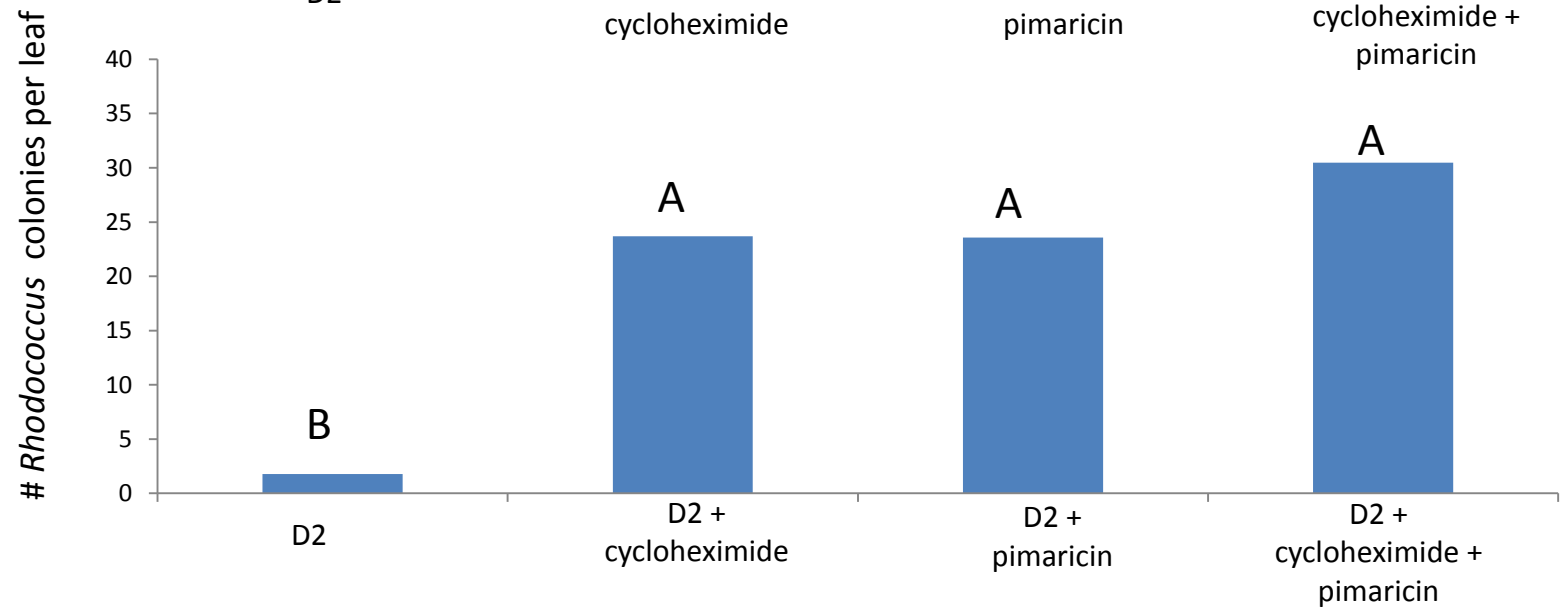
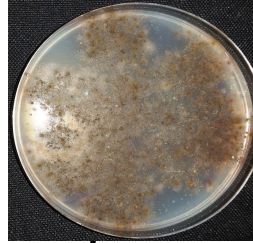
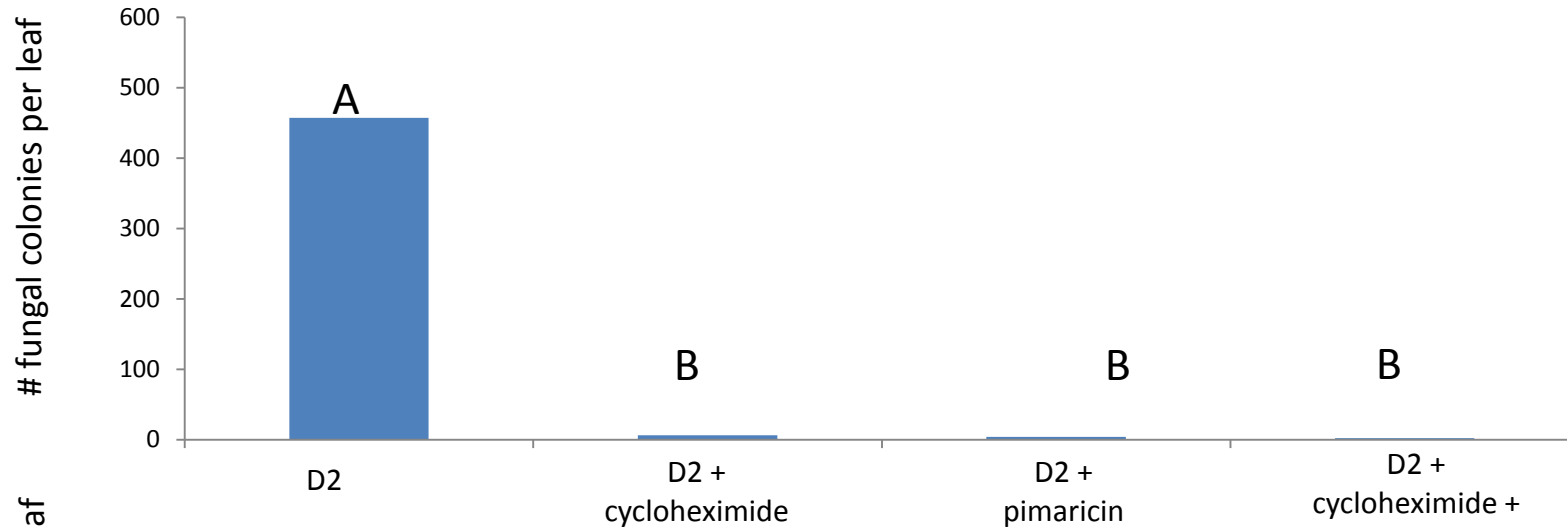


D2+Cycloheximide + Pimaricin

Leaf Prints from
symptomatic PBTS
rootsock

Dhaouadi and Fichtner,
unpublished data

Amendment of D2 medium for enhanced detection of *Rhodococcus* spp. on PBTS trees



Data combined from two runs; N=11

Glyphosate injury on prune

Photo: Dr. Brad Hanson, CE Specialist



Are *Rhodococcus* spp. associated with all symptomatic plants?

Glyphosate injury on almond



Glyphosate injury on almond



www.calpistachioresearch.org



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Pistachio Bushy Top Syndrome (PBTS) - what you need to know*

[PBTS - Questions and Answers \(Robert E. Klein\)](#)

[PBTS - Research Report \(Jennifer J. Randall\)](#)

[PBTS - Link to Pistachio Day presentation by Jennifer J. Randall](#)

[PBTS - Kearney PBTS Workshop Presentation \(PDF\)](#)

*This webpage will be used to post PBTS information updates - last updated 3/2/2015.

Are there replant issues associated with PBTS?







- *Rhodococcus* spp. isolated from roots of PTBS trees.
- Low recovery frequency (prior to new media)
- Origin of inoculum unknown.

Are replant soils infested with *Rhodococcus* spp?



- PCR positives from replant soil (Randall laboratory). Viability and source of inoculum unknown.

- Isolation from replant roots (February 2015) (Fichtner)

Replants: Unaffected nursery source; planted in former bushy top holes; July 2014-February 2015.

Fresno Co.




Original source inoculum?



Methodical assessment of replant roots during 2015 growing season.

Roots of replants in PBTS-affected sites were methodically sampled in 2015 to determine presence of *Rhodococcus* spp.

Site Location	Destructive vs. non-destructively sampled	Month of sampling	Sample Size	Time of PBTS orchard removal	Time of orchard replant	Isolation of <i>Rhodococcus</i> spp? Frequency of isolation?
Fresno Co.	Destructive	February 2015	6	July 2015	July 2015	Yes- one out of 6; orange colony type 
Kern Co.	Non-destructive	September 2015	10	2014	2014	None
Kern Co.	Non-destructive	October 2015	20	January 2015	February 2015	None
Kings Co.	Destructive	September 2015	10	September 2014	October 2014	None

- Generally not isolated from replant roots in the field.
(Good news)

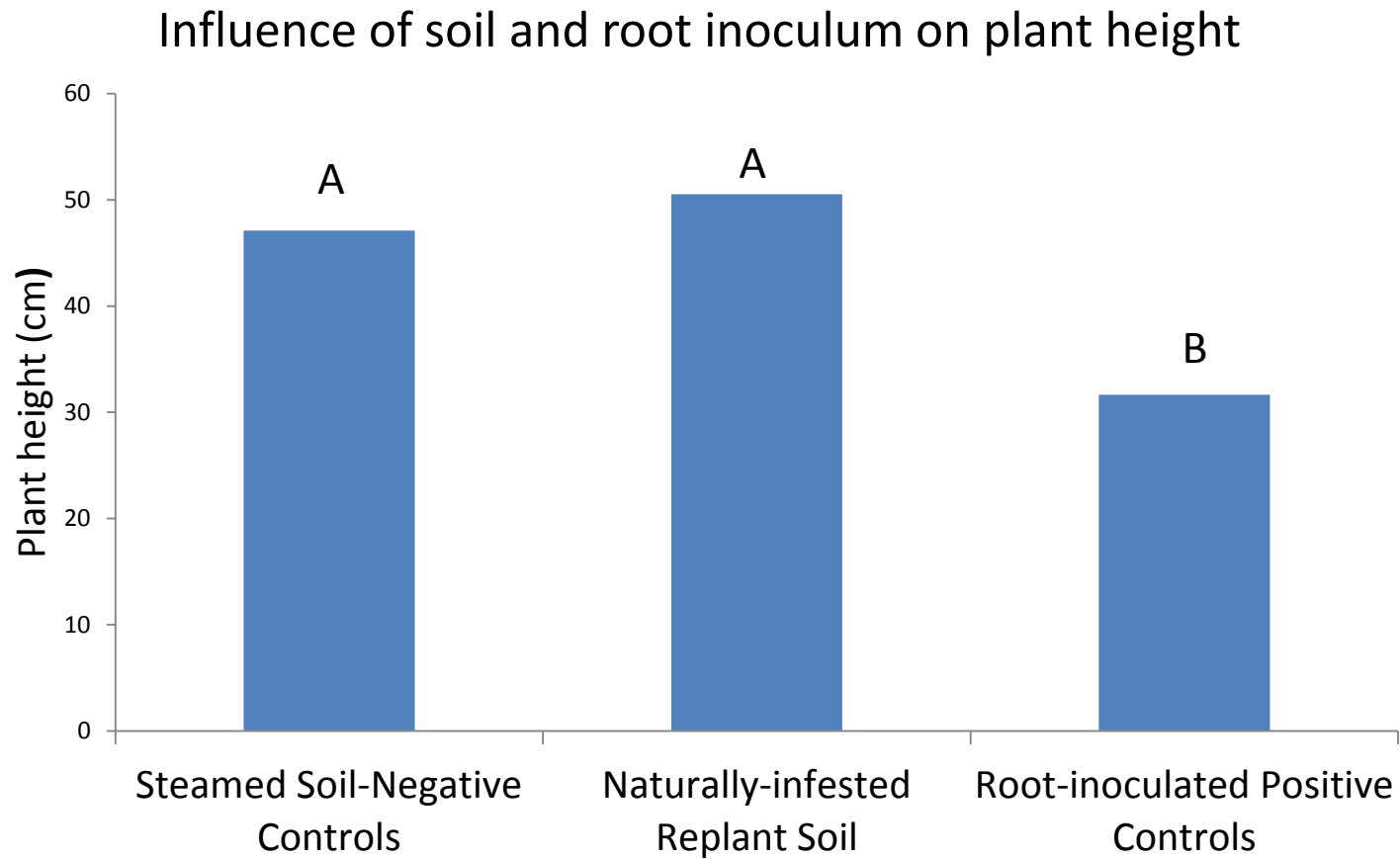
Replant Study:

Steamed soil= negative control

Inoculated roots=positive control

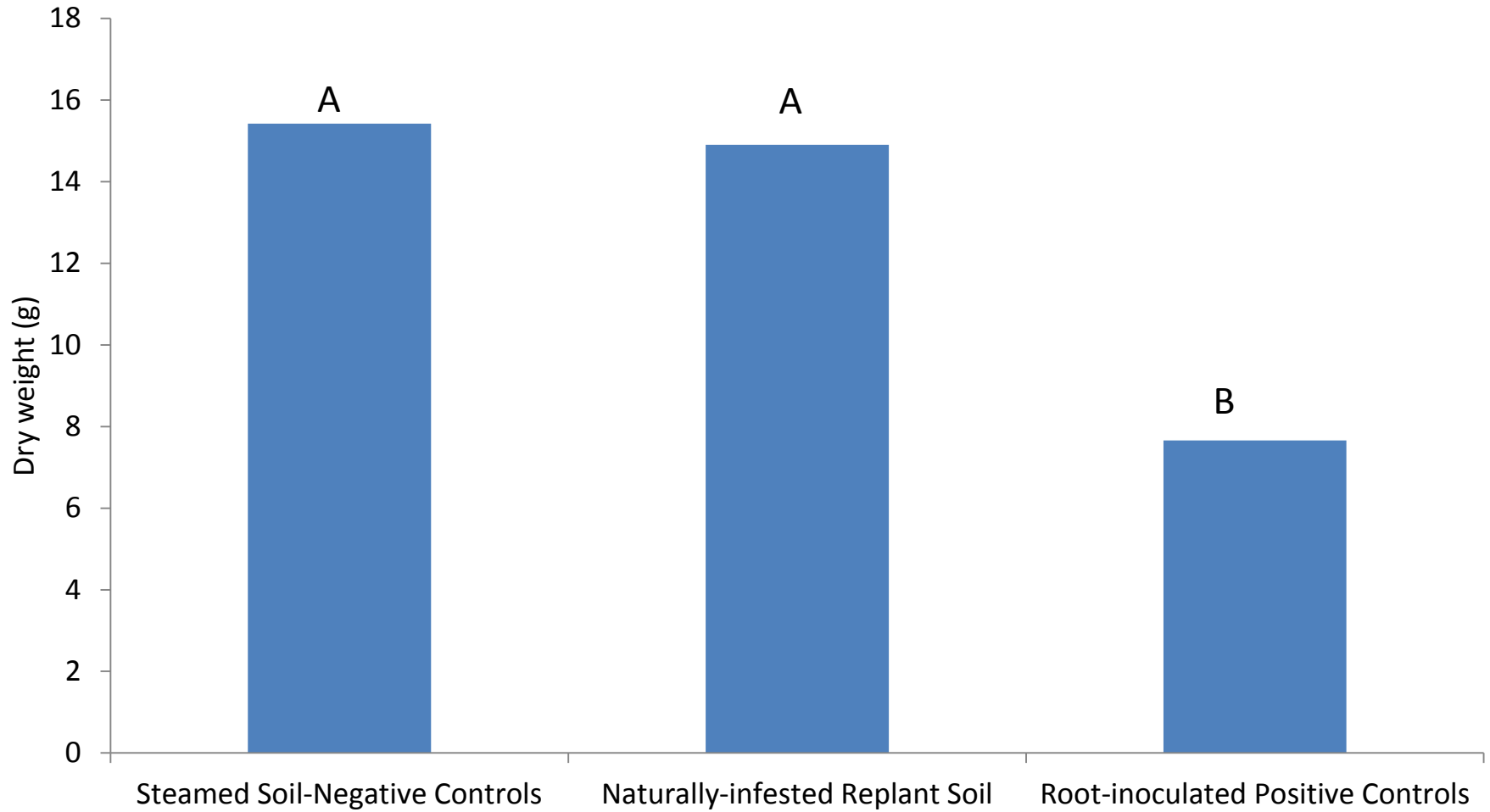


Replant Study: 11/25/2014-5/27/2015



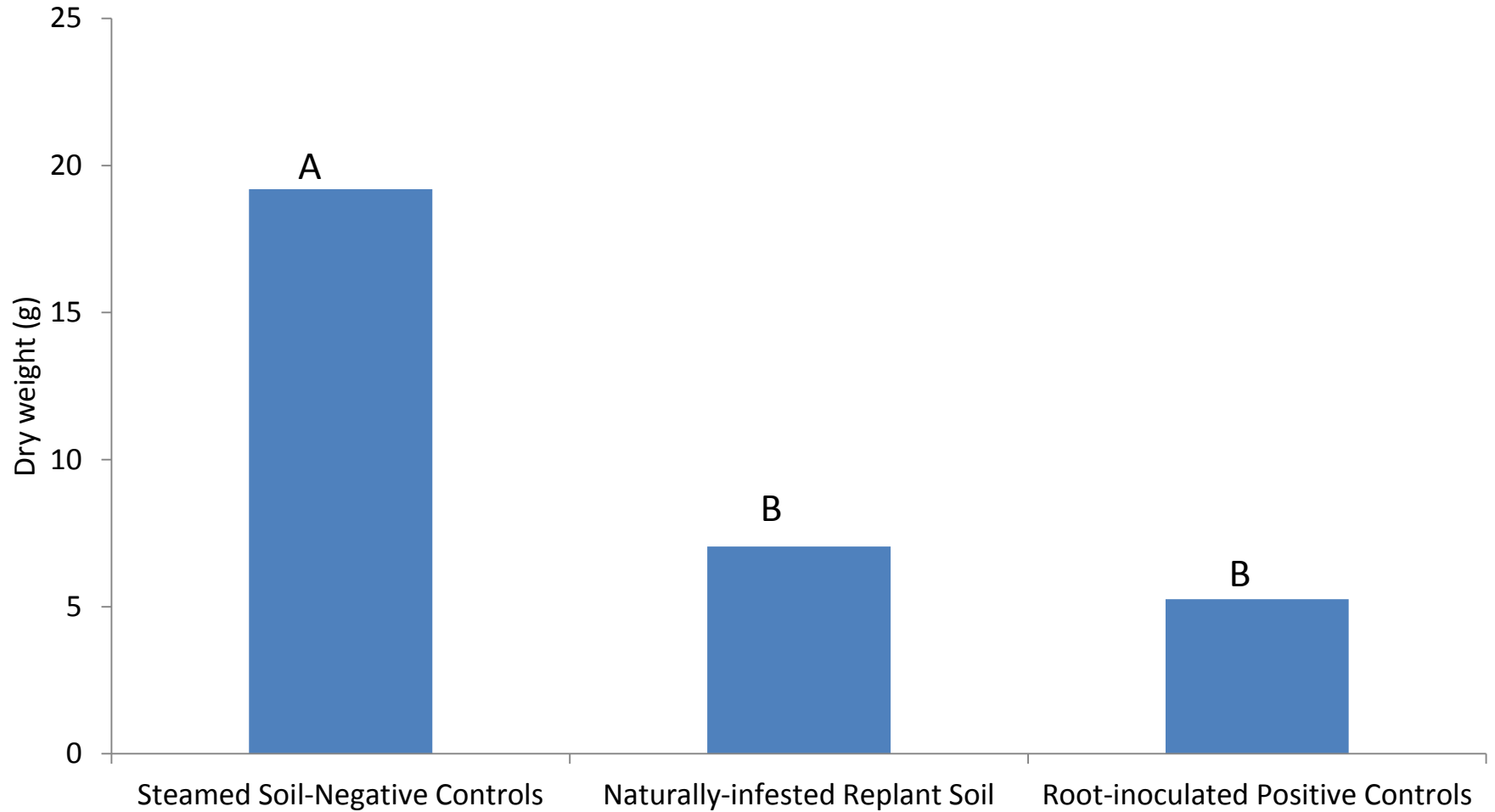
Replant Study: 11/25/2014-5/27/2015

Influence of soil and root inoculum on shoot biomass



Replant Study: 11/25/2014-5/27/2015

Influence of soil and root inoculum on root biomass





Positive Control UCB-1 roots
inoculated with PBTS *Rhodococcus*
isolates

Isolation Recovery of *Rhodococcus* spp.

	Uninfested Control	Naturally Infested	Inoculated (+) Control
Root Press	0/18	7/18	18/18
Root Grindate*	0/18	4/18	9/18
Total (press+ grind)	0/18	9/18	18/18

* Three 1cm root segments sampled per plant. Not surface sterilized. Ground in sterile di water and plated 200uL of suspension.

Almond Rootstock ('Hansen') inoculated with PBTS isolates of *Rhodococcus* spp.

'Hansen' (Nemaguard peach x almond)







Uninoculated Control

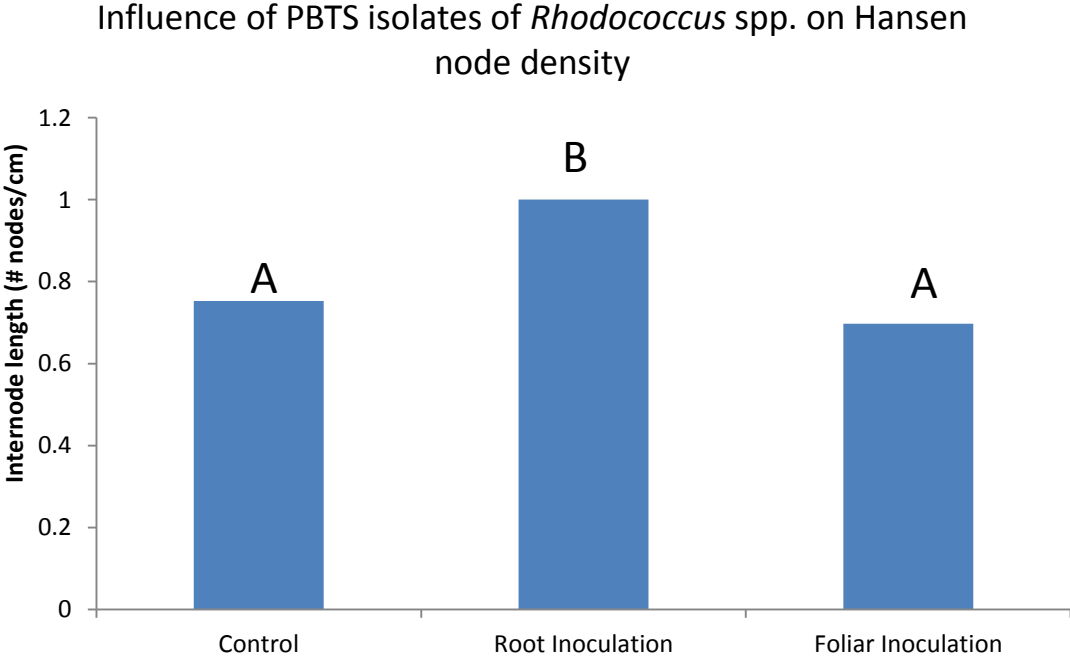
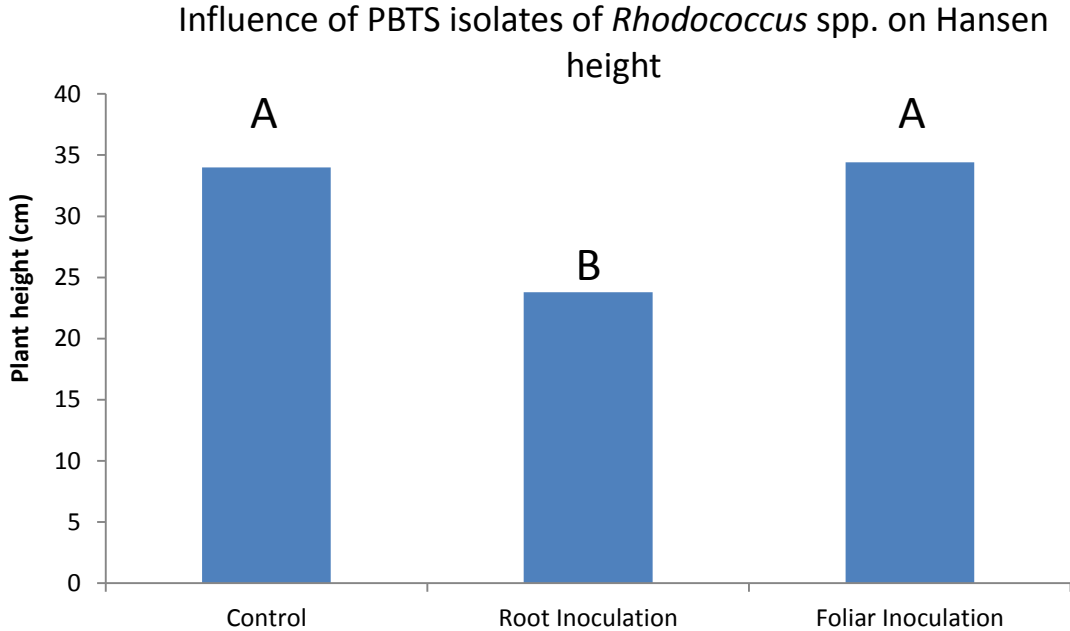
Inoculated Roots

Hansen Rootstock

ANOVA	P value
height	0.01 
# nodes	0.69
# leaves	0.1892
# nodes/cm	0.0001 
# lvs/node	0.3135

Hansen rootstock: susceptible to root inoculations with PBTS isolates of *Rhodococcus* spp.

Dhaouadi and Fichtner, unpublished data



How long do *Rhodococcus* spp. survive in California orchard soils?

Field trial: Vanguard sandy loam, 6" vs. 30" depth.



Controlled study (environmental conditions):

Vanguard sandy loam vs. Gambogy-Biggriz saline-sodic

Container Capacity (irrigated) vs. Drying

Rhodococcus fascians vs. *Rhodococcus corynebacterioides*-like





Gravimetric Soil Moisture @ Container Capacity

Vanguard : 29%

Gambogy-Biggriz: 32%

Soils at container capacity vs. drying

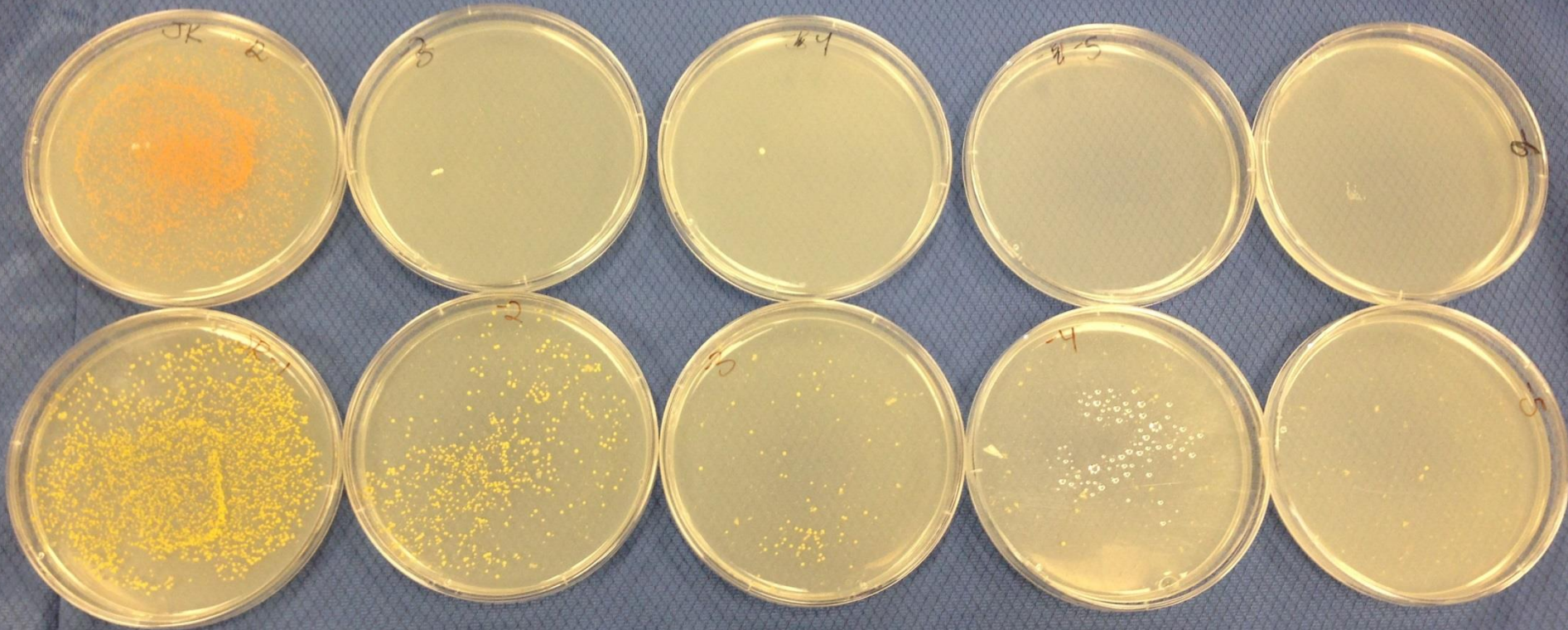


Inoculum embedded on toothpicks



Dilution Plating to quantify inoculum

***Rhodococcus corynebacteriodes*- like**
 5.2×10^4 cfu/stick



Rhodococcus fascians
 1.2×10^5 cfu/stick

Goal: Determine survival potential of PBTS isolates of *Rhodococcus* in CA pistachio orchard soils.

- Environmental influences on survival:
 - soil moisture
 - organic matter
 - season
 - depth
 - soil type



Can *Rhodococcus* be transmitted on infested pruning shears?

3 Treatments (N=20)

Surface-disinfested shears

Naturally-infested shears

Artificially-infested shears (yellow + orange isolates)

Timeline (6+ months)

Treatments initiated 11/24/14

Leaf press: 4/2/2015 (newsletter)

Leaf press: 6/8/2015

Surface sterilized leaf grindate: 6/10/2015

Plant height/shoot and root weight: 6/10/2015



Reisolation from pruning-transmission experiment

	Negative Control	Naturally-infested	Artificially infested
June Leaf Press	1/20*	13/20	15/20
June Leaf Grindate (surface sterilized)	NONE	NONE	NONE

- Yellow and Orange isolates both recovered with similar frequency
- **No endophytic populations detected.**
- No statistical difference in plant height or shoot and root mass.

* A single colony was found on negative control leaves.



CCU



Fresno County, October 2014

Stem Cracking on walnut: seedling PDX



Rhodococcus fascians positive

Yellow isolate

(Randall laboratory)

Leafy gall on rootstock

R. fascians

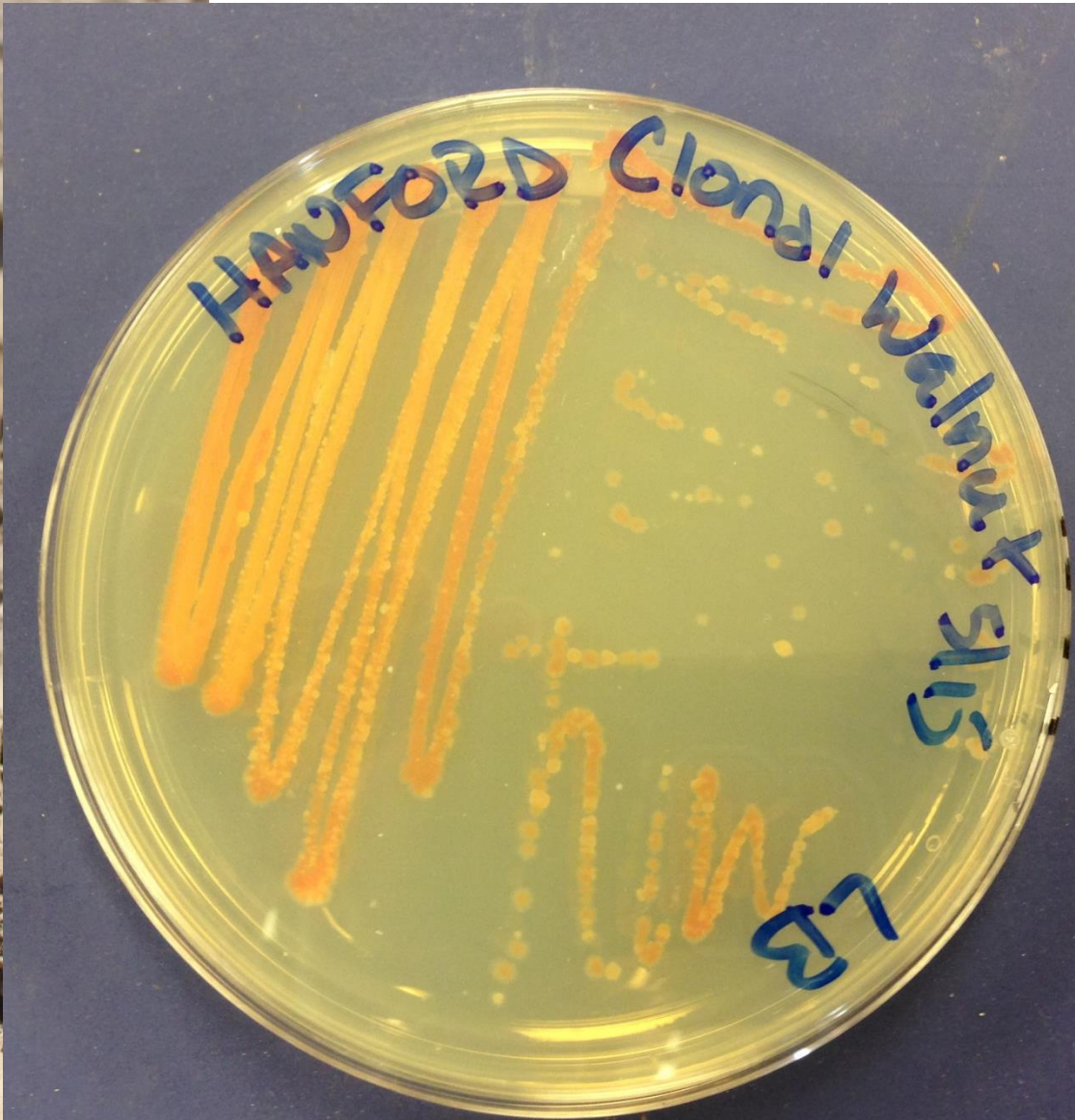
Orange and Yellow
colonies
(Fichtner)



Yolo County; April 2015



Kings County: May 2015





How prevalent are *Rhodococcus* spp. on California walnuts?

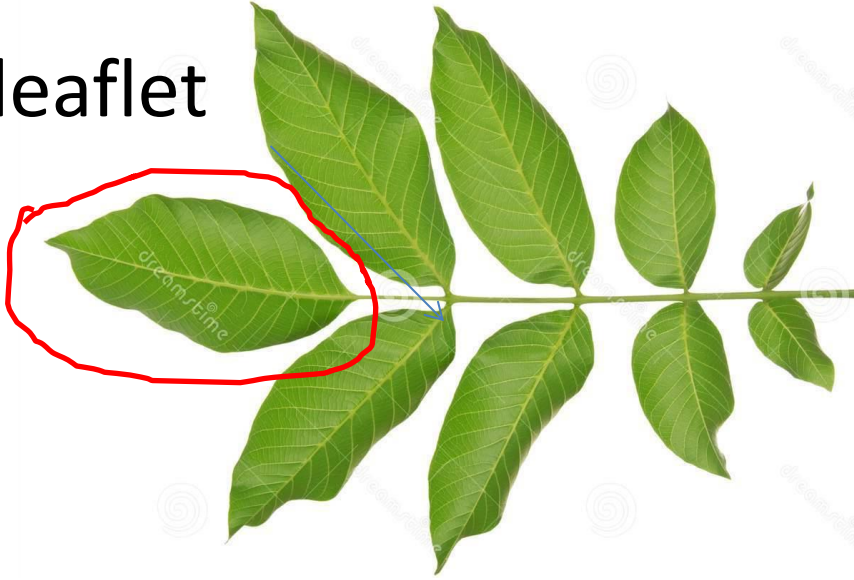
Yu, X., Liu, X., Zhu, T.H., Liu, G.H, Mao, C. 2011. Isolation and characterization of phosphate-solubilizing bacteria from walnut and their effect on growth and phosphorus mobilization. *Biology and Fertility of Soils*. 47:437-446.

- *Rhodococcus* sp. found in rhizosphere soil of *Juglans* in China.
- *Rhodococcus* not found CA walnut crown tissue (LPC survey) (Browne, personal communication)



2015: *Rhodococcus* survey on asymptomatic English walnuts

leaflet



County	# Orchards	Variety
Tulare	12	Tulare, Chico, Serr, Vina, Chandler
Fresno	3	Tulare, Chandler, Serr
Madera	1	Chandler
Stanislaus	3	
TOTAL	19	

Standardized Collection Procedure

4 Counties
19 Orchards
5 Varieties

Standardized Detection Procedure

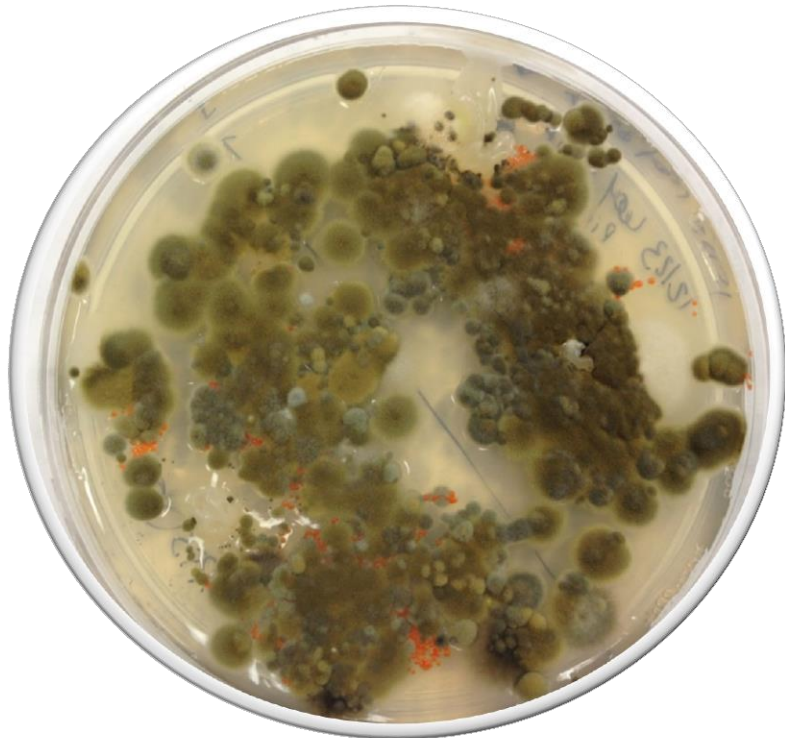
Epiphytes
Endophytes

Collaborators: G. Brar, K. Kelley-Anderson

***Rhodococcus* spp. NOT detected as epi- or endo-phyte in asymptomatic orchards**

20/20 Hindsight

This study was conducted BEFORE we improved our selective medium for pathogen detection.



Rhodococcus spp. HAVE been detected on asymptomatic plants neighboring symptomatic plants.

Yolo County



Fresno County

E Clarkson Ave

Symptomatic

S Cedar Ave

Symptomatic

New planting; sampled 4/20/15



Prevalence of *Rhodococcus* spp. on symptomatic and asymptomatic tissues in SAME orchard

December 2015

20 symptomatic trees
20 asymptomatic trees

Endophytic only
(surface disinfested in field)

Asymptomatic Trees

1/20 Putative *Rhodococcus*

Symptomatic Trees

9/20 Putative *Rhodococcus*



1. *Rhodococcus* isolates from walnuts w/ odd morphologies.
2. Yellow and orange isolates.
3. Plasmid detected.
4. Plants sourced from multiple nurseries.
5. Clonal and seedling rootstock and scion material.
6. No association with pistachio bushy top syndrome.

Pathogenicity on walnut unknown

'Present but not prevalent' on walnut

Proposal to CA Walnut Board for completion of Koch's postulates in 2016

PBTS isolates on English walnut seedlings-in progress.

Rhodococcus spp. studies in progress

- Replant study
- Pruning transmission
- Mouse transmission
- Soil survival
- Walnut Susceptibility
- Prevalence on symptomatic walnut (CSU Bakersfield)



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CSU Bakersfield

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